

MHC Evolution in Rockfishes

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The major histocompatibility complex (MHC) is a multigene family found in vertebrates. The MHC contains genes that are involved in the processing and presentation of antigens to cells of the immune system, and are critical to any cell-mediated immune response. MHC class II genes are specifically responsible for the presentation of exogenous or extracellular antigens to the immune system. Genetic variation at MHC class II genes is known to be important in pathogen resistance and may also be important in mate choice. The MHC has only been studied in a relatively small number of fish species, and has not yet been looked at in rockfishes (*Sebastes* spp.). Isolation of MHC genes in rockfishes will provide insight into the genomic organization of MHC genes in higher teleosts, general modes of MHC evolution in fishes, and a valuable tool for the study of selection, mate choice, and population genetics in rockfishes. Our objectives are to 1) isolate MHC class II genes from a variety of rockfish species, 2) perform phylogenetic analysis of rockfish and other teleost MHC sequences, 3) determine whether nonsynonymous substitution rates are elevated compared to synonymous substitutions, and 4) test for locus-specific amplification.

Rockfish tissue samples were obtained from NMFS trawls and longline collections. DNA was extracted using standard protocols. Polymerase chain reaction (PCR) primers were designed to amplify a portion of the MHC class IIB gene, from intron 1 to exon 2 (~500 base pairs [bp] fragment). PCR products were then subcloned and sequenced. An additional set of PCR primers were designed based on intron 1 sequence, and amplified on six greenspotted rockfish (*S. chlorostictus*) and striptail rockfish (*S. saxicola*) individuals to test for locus-specific amplification. Phylogenetic analysis and estimation of nonsynonymous (amino acid replacement, d_n) and synonymous (silent, d_s) substitution rates was done with MEGA2 (Kumar 2001) for exon 2 fragments only.

MHC class IIB gene fragments were isolated from six rockfish species: aurora rockfish (*S. aurora*), blackgill rockfish (*S. melanostomus*), copper rockfish (*S. caurinus*), greenspotted

rockfish, halfbanded rockfish (*S. semicinctus*), and striptail rockfish. Multiple loci were that phylogenetic isolated from each of the species. Some sequences contained frameshift mutations, leading to an incorrect open reading frame, indicating that pseudogenes may be present in the rockfish MHC. Figure 1 shows analysis revealed a distinct cluster of rockfish MHC genes when

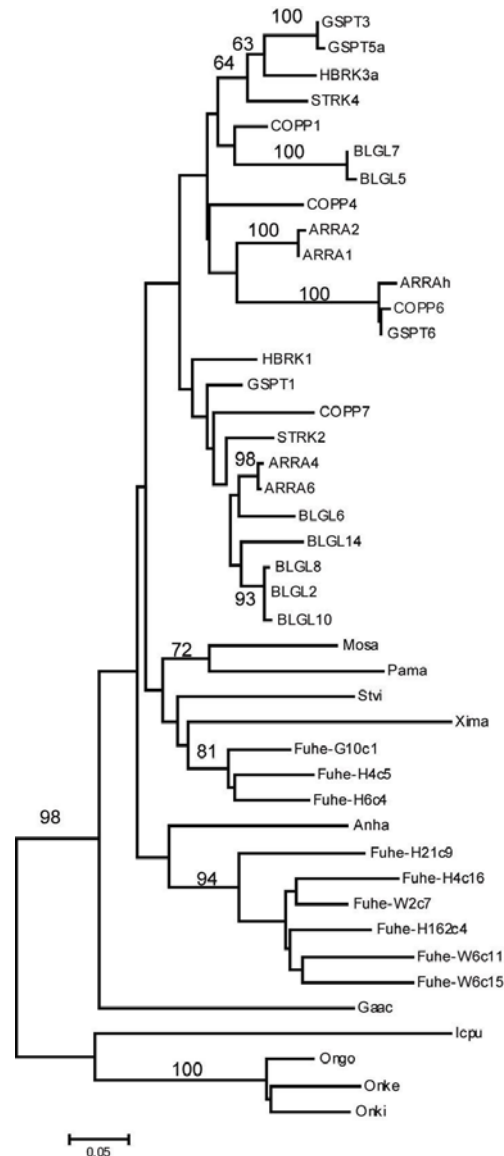


Figure 1. Neighbor-joining tree, estimated using the Tamura-Nei distance measure, for all rockfish MHC class IIB alleles (exon 2) and MHC alleles from other teleost species. Numbers next to nodes indicate percentage of support from 500 bootstrap replicates. Species codes follow. Rockfishes: ARRA: aurora rockfish; BLGL: blackgill rockfish; COPP: copper rockfish; GSPT: greenspotted rockfish; HBRK: halfbanded rockfish; STRK: stripetail rockfish; nonrockfish species: Anha: *Aulonocara hansbaenschii*; Fuhe: mummichog (*Fundulus heteroclitus*); Gaac: threespine stickleback (*Gasterosteus aculeatus*); Icpu: channel catfish (*Ictalurus punctatus*); Mosa: striped bass (*Morone saxatilis*); Ongo: pink salmon (*Oncorhynchus gorbuscha*); Onke: chum salmon (*O. keta*); Onki: coho salmon (*O. kisutch*); Pama: red seabream (*Pagrus major*); Xima: southern platyfish (*Xiphophorus maculatus*); Stvi: walleye (*Stizostedion vitreus*, now *Sander vitreum*).

Table 1. The nonsynonymous (d_n) and synonymous (d_s) substitution rates for the MHC class IIB alleles isolated from greenspotted rockfish and stripetail rockfish and all rockfish species used in this study. Standard errors are given in parentheses.

Species	d_n	d_s	$d_n:d_s$
Greenspotted rockfish	0.187 (0.036)	0.035 (0.013)	5.34
Stripetail rockfish	0.175 (0.033)	0.056 (0.019)	3.13
All rockfish	0.299 (0.038)	0.086 (0.024)	2.66

compared to other teleost MHC sequences. Transspecific allelism, indicative of a history of balancing selection, is evident in the comparison of *Sebastes* MHC alleles (Figure 1). Redesigning of primers based on intron 1 sequence data gave locus-specific amplification for two of the species: greenspotted rockfish and stripetail rockfish. Elevated $d_n:d_s$ ratios were found for a comparison of all rockfish MHC genes (Table 1) as well as for the alleles isolated from greenspotted rockfish and stripetail rockfish.

The number of MHC loci appears to be variable across rockfish species. This is evident by the number of unique sequences that were isolated from each of the rockfish species. This finding of multiple MHC class II loci within rockfish has also been found in other teleost species (e.g., cichlids [Cichlidae] and sticklebacks [Gasterosteidae]). Elevated $d_n:d_s$ ratios indicate that positive selection has been operating on rockfish MHC sequences, demonstrating historical selection and possible functionality to the genes isolated. Preliminary results suggesting locus-specific amplification in two species (greenspotted rockfish and stripetail rockfish) gives hope for further molecular evolutionary and population genetic studies of MHC class IIB genes in rockfishes.

This is a work in progress, as we are continuing in the isolation of MHC sequences from a larger number of rockfish species. We also plan to verify locus-specific amplification through mother-offspring comparisons. We are also establishing genotyping methods (i.e., single strand conformational polymorphism, or SSCP) for MHC in rockfishes to conduct population-level studies of MHC variation in rockfishes.

References

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Conservation of North Pacific Rockfishes: Ecological Genetics and Stock Structure

Proceedings of the Workshop
March 2–3, 2004
Seattle, Washington

Edited by Ewann A. Berntson, Phil S. Levin,* and Paul Moran

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April 2007

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
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